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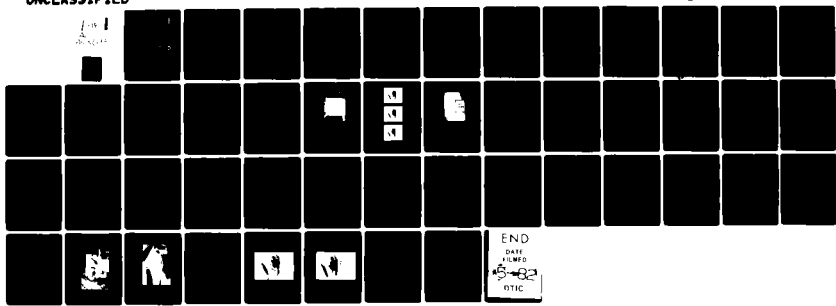
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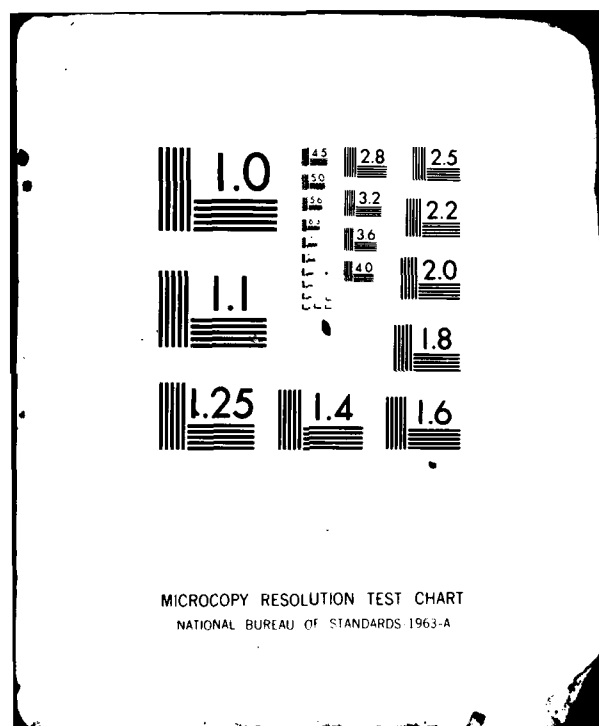
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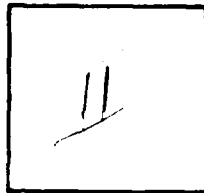
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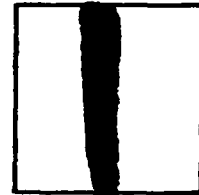
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RESEARCH PROGRAM ON THE ULTRASONIC IMAGES OF TISSUES

AD A11 3035

FINAL REPORT

W. D. McNeely

and

Abraham Noordergraaf, Ph.D.

August 1980

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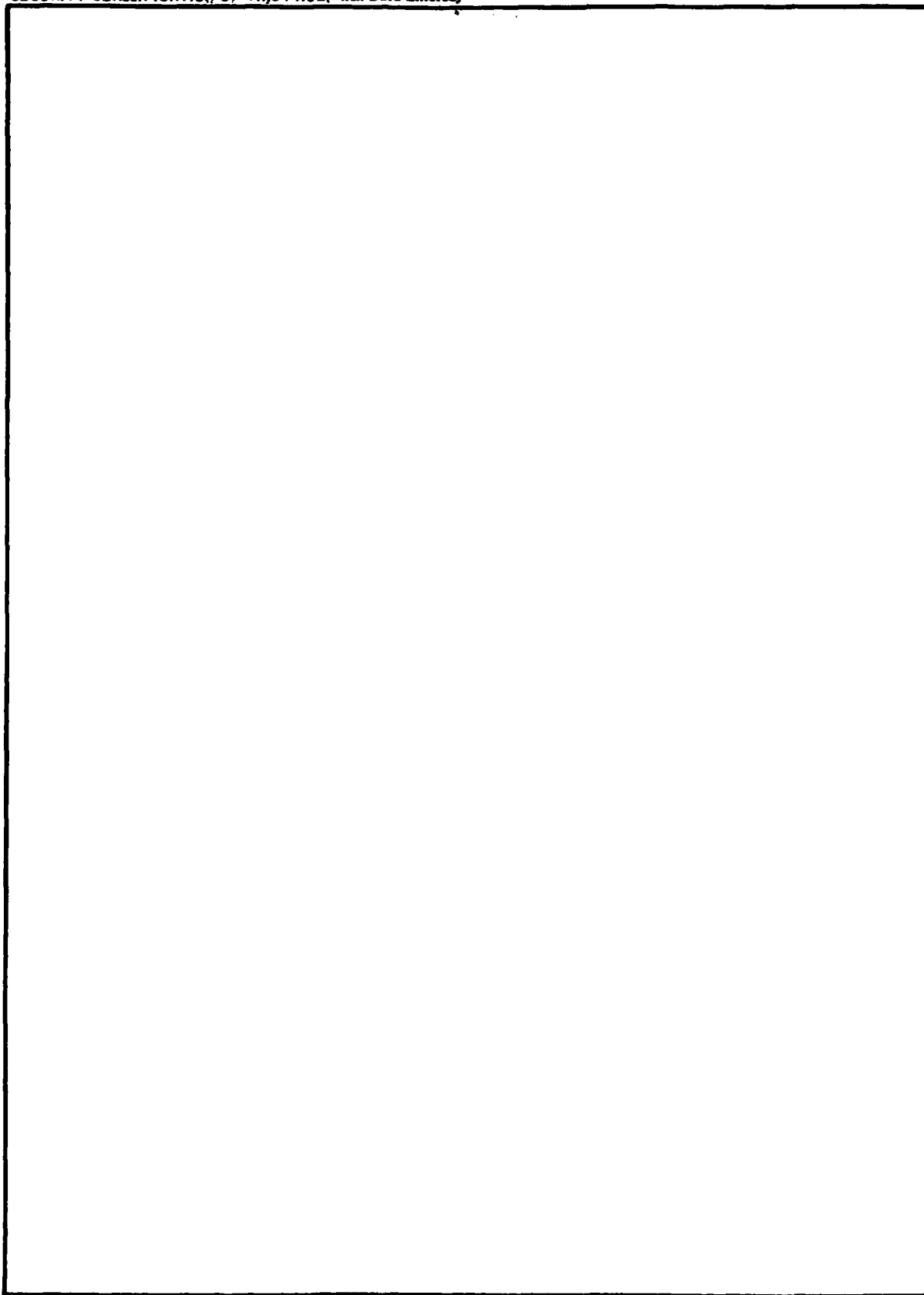
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Summary

The purpose of this study was to determine whether measurable changes occur in acoustic properties of muscle during the first several hours after death.

Cats were chosen as the experimental animal for their size, availability, and ease of handling. Sodium pentobarbital was used for anesthesia. Utilizing an instrument called Ultrasonovision, ultrasound attenuation was measured of an area of muscle between the patella and the fibula in five cats at 1.75 MHz.

The measurements indicate that from living to four hours post-mortem attenuation decreased steadily. This change is statistically significant. In subsequent hours attenuation appeared to increase again. Not enough data points are available to determine the statistical significance of the latter.

It is concluded that there are at least two mechanisms affecting acoustic attenuation during the immediate hours following death. These mechanisms have yet to be identified.

Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

The ultrasonic measurement equipment utilized in this study was assembled by RCA/Princeton and the necessary expertise was transferred under a subcontract.

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Introduction

This report represents the progress on the third and final phase of the research program funded by the U. S. Army Surgeon General's Office, Contract DAMD-17-74-C-4107. The main thrust of the program was to determine whether ultrasound would be able to differentiate between living and dead tissue. The term dead refers to tissue that is physiologically dead. In the treatment of traumatic wounds such as those resulting from gunshots, accidents, etc., it would be helpful for the attending physician to know the extent of tissue damage. This determination could be done quickly and non-invasively using ultrasonic methods. Therefore, it is important to determine if ultrasonic imaging can detect the difference between healthy and damaged tissue.

The research program was divided into three phases, each of about one year in length. The first phase dealt with physical models that were designed to help define the resolution and imaging characteristics of the RCA Ultrasonovision.³ See Figure 1 for a diagram of the Ultrasonovision. Results of Phase I showed that the Ultrasonovision could resolve objects as small as 1 mm in cross section (approximately the diameter of the laser beam used in the detection system) and that the dynamic range was large enough (40 dB) such that a large range of attenuating objects could be imaged.

Phase II was concerned with determining the characteristics of dead tissue in vitro. Bovine tissue was selected due to its availability and the fact that large fat-free specimens could be obtained. A local abattoir was able to supply muscle tissue within several hours of sacrifice of the animal, thereby providing a reasonably close link to living tissue.

Samples of the bovine tissue were mounted as shown in Figure 2 and then scanned ultrasonically at regular intervals for a period of two days following the sacrifice of the animal. Data collected from the experiments indicated that acoustically, the tissue characteristics (in particular attenuation) changed as the tissue aged.⁴

The results of Phase II were encouraging in that they provided a positive indication that, using ultrasound, small changes in tissue state via changes in tissue attenuation could be detected. This led to Phase III since, if differences in tissue age after death could be detected with ultrasound, then differences between living and damaged tissue should also show up as a change in attenuation.

Experimental Procedure

Since Phase III was to involve in vivo measurements, an experimental animal had to be found that fit a number of requirements: the animal had to be small for ease of handling, since the animal had to be mounted in a water bath and, second, the animal had to be readily available. Dogs were eliminated mainly because of the size of so-called "common" varieties. The cat was the only animal that offered both small size and ease of availability, plus the cat had a reasonably large muscle area on its hind leg.

As indicated in Reference 3, any object that is to be imaged by the Ultrasonovision has to be immersed in a water bath. Therefore, to hold the cat in an upright position in the water bath for imaging purposes, a rack was designed and built (Figure 3). In addition to holding the cat in an upright position, the rack assembly allowed the animal to be moved in and out of the ultrasound beam without changing the axial location,

i.e., distance from animal to pellicle. This was very critical since the recorded picture was a composite of the entire leg with emphasis on the focal plane of the acoustic lens system. Exact positioning was maintained to insure that image changes occurring during the experiment were due to tissue aging and not a relocation of the animal.

The animal was first anesthetized using sodium pentobarbital; the hind legs were shaved, using standard animal clippers, and then the animal was strapped to the rack assembly as shown in Figure 3. A plastic bag of 1.5 mil polyethylene was placed around the lower assembly of the rack and the hind legs to prevent contamination of the tank. The 1.5 mil polyethylene did not attenuate the ultrasound since it was acoustically very well matched with water. One of the hind legs was positioned normal to the axis of the ultrasonic beam, with focusing on the area of the muscle between the patella and the fibula. After insertion of the animal into the tank, the leg to be scanned was carefully rubbed to remove air bubbles which would block the ultrasound, causing artifacts to appear on the image and, hence, excessive attenuation values.

A preliminary acoustic image was taken to provide a map for the sites of interest (see Figure 4). Usually sites of both high and low attenuation (extremes) were chosen. An initial attenuation reading was recorded at each of the preselected sites on the live animal; then an overdose of sodium pentobarbital was administered to terminate the animal. The time of the overdose was recorded as the time of death, and all subsequent readings are referred to as "post-mortem." Each hour thereafter, the attenuation of each site was remeasured. Each reading consisted of recording the ultrasonic intensity with the animal in the beam, and a

reading with the animal out of the beam. For a single location, twelve readings were taken through the tissue and twelve along the reference path. Thus, approximately one hour was required to make the measurements for six different locations. Twelve readings were necessary at each spot due to the inherent instability of the system (e.g., any vibration of the environment directly affects the intensity reading by randomly changing the amount of light that reaches the detector). Small vibrations are normally overcome by the "wiggler," however, in the present laboratory, the vibrations were of such magnitude they tended to override the wiggler movement. Spatial noise from dirt on the lens and pellicle also contributed to the instability of the system by causing varying degrees of reflection from the pellicle face and scattering of light as it passes through each lens. The problem of instability is now in the process of being eliminated on the system at the Indianapolis Center for Advanced Research. With such improvements, the Ultrasonovision system should be usable for attenuation measurements to ± 0.1 dB accuracy.

The attenuation coefficient (directly related to the absorption coefficient) is derived from experimental data by measuring the clear path signal intensity and the tissue path intensity. The attenuation is given by the following equation:

$$\text{Attenuation} = 20 \log \frac{\text{tissue path intensity}}{\text{clear path intensity}} .$$

Attenuation was determined without identifying the individual components that make up the total "attenuation;" i.e., scattering, reflection and

absorption. The various components were to be separated and identified in later experiments.

Several parameters remained fixed throughout the duration of each experiment. The water bath temperature was maintained at 38°C (i.e., approximately the body temperature of a cat). The focal point of the acoustic lens-transducer system remained at the same point in the tank. This was checked before each experiment in two ways. First, an acoustic image of a focusing grid was generated and second, the actual intensity difference between holes and metal of the focusing grid was maximized. The left side of Figure 5 shows an optical image and the right side of Figure 5 shows an acoustic image of the focusing grid. The same transducer excited at 1.75 MHz was used throughout the experiments.

To the original RCA system, i.e., the system used in Phases I and II, a spot reading device was added. This device is capable of making an accurate intensity reading on a single pixel of an entire scan. Measurements are repeatable due to the fact that the spot recorder is equipped with a calibrated X-Y grid system. Thus, accurate relocation of each spot of interest is possible over a long period of time, i.e., hours.

The experiment ran for a total of 6 to 7 hours. Usually, termination of the experiment was dependent upon the state of the animal (i.e., the faster the decomposition, the shorter the experiment).

Results

Upon completion of the first animal, the attenuation values were calculated for each spot. These values were then plotted on the graph shown in Figure 6. Preliminary analysis indicated that the data followed the pattern predicted by Phase II; that is, with increasing time from

death, the attenuation decreases. The experiments were then continued through five additional animals. Plots of four animal results are shown in Figures 7, 8, 9, and 10. Figure 11 shows a composite plot of all the data from points of approximately equal attenuation. The bars indicate standard deviation limits, while the small dots indicate the mean value. The overall data indicates that the standard deviation is entirely too large to allow a definite conclusion. The experiment on animal number 2 was not completed, as the animal expired prior to the measurement of the attenuation on the living tissue; therefore, no data was recorded for that animal. As can easily be observed from the plots in Figures 7-10, none of the animals appeared to follow the pattern of animal number 1. To confirm this fact, a statistical analysis was done on the composite data. Variance relationships were checked between cats; between cats and time, between cats, time and location, and between cats and location of the spot reading (i.e., high or low attenuation).

Conclusions and Discussion

The only significant relationship found was that there is a statistical difference between cats. All other changes were found to be insignificant. Of course, the difference between cats was obvious and did not add any information to the experimental results. Therefore, it can be stated that with the present experimental equipment and procedure, there is not a detectable difference between living and dead tissue using ultrasound as the imaging medium. A number of variables exist that may or may not change the outcome of the original hypothesis.

First, to calculate the normalized attenuation (dB/cm), a thickness measurement has to be made on the animal's leg where it was scanned. In

the present experiment, these measurements were made on the leg using a micrometer.

If the leg was found to be approximately 2 cm thick, then a 1 mm error caused by compressing the skin slightly would result in a 5% error in the attenuation value. Thickness readings were taken prior to the beginning of the experiment. If, due to edema of the leg after death, there was a 1 to 2 mm increase in the thickness, another source of error would be introduced. Thus, in the future, a more accurate method must be found to measure the sample thickness to eliminate such errors.

Preparation of the animals' skin surface as mentioned earlier consisted of shaving off the hair and, upon insertion into the water bath, the leg was rubbed to remove air bubbles. An improved method would be to use a depilatory after shaving to completely remove all the hair. This would eliminate any possibility of air being trapped at the skin surface.

Another area that needs to be investigated is whether or not dead tissue is different pathologically when death occurs naturally or with trauma (i.e., accident, etc.). For example, the bovine tissue obtained in Phase II came from an animal that was sacrificed with a blow to the head. The tissue from these animals changed in attenuation in a consistent pattern. Perhaps the pathological state of the bovine tissue was different from that of the cat due to the method of sacrifice and/or the method of preparing the tissue. The bovine tissue was excised, then mounted, so it was exposed to foreign matter (i.e., water, saline solution); whereas the cat tissue was not removed from the animal and the skin remained intact.

Correlation of the acoustic image to the actual physical specimens is another problem area. Dark spots (high attenuation areas) need to be correlated to some particular type of tissue, and the same applies to white or less attenuating areas. After scans of the leg muscle are made, careful sectioning of the imaged area should be done so as to reveal the origins of the features of the acoustic image.

A better method of holding the animal in place is necessary. The present system uses straps around soft tissue to support the animal; then, after termination of the animal, sagging results due to relaxation of the muscles, thus changing the position of the imaged area. Either a better method of strapping needs to be devised, or use of acoustic markers applied to the leg to provide continuous correlation points.

Finally, the method of detection of changes in tissue state must be revised. Recent experiments by Lele¹ and Franklin² have shown that, using backscatter, very small changes in tissue pathology may be detected. This is possible due to the relative magnitude of the signals. The differences in tissue pathology are due to alterations in structure rather than molecular decomposition; hence, the changes will affect backscatter rather than the absorption coefficient. The RCA Ultrasonovision of the University of Pennsylvania is not capable of making measurements of the level necessary to record backscatter. Presently at the Ultrasound Research Laboratories of the Indianapolis Center for Advanced Research, an RCA Ultrasonovision is being modified to detect low level signals. When the modifications are completed, an additional set of experiments will be undertaken to test the results of Phase III.

Recommendations

The ultimate goal of these experiments was to evaluate ultrasound as a means of non-invasively detecting normal and damaged tissue. A non-invasive method would be an invaluable guide for a surgeon treating battlefield wounds that would determine which tissue must be removed from the damaged area. Both Lele¹ and Franklin² have used ultrasound to detect ischemia in the myocardium of experimental animals. Thus, it should be possible to detect damaged tissue in other parts of the body.

The present experiments were not able to detect differences between normal and tissue damaged beyond recovery with any sort of consistency. This inability could have been the result of the method of detecting changes in the tissue state. The method used in the present experiments was to measure the intensity of the transmitted ultrasound after it passed through the tissue sample. If the changes in tissue state were very small compared to the overall magnitude of the ultrasound intensity, then the changes would be difficult to measure due to the inherent instability of the RCA Ultrasonovision. A better method of detecting small changes would be to compare these changes to small signal levels such as those from the backscatter^{1,2} or the first order diffraction image.^{5,6}

Presently at the Ultrasound Research Laboratories of the Indianapolis Center for Advanced Research, an RCA Ultrasonovision is being modified to enable measurements to be taken of very low level signals resulting from backscatter or diffraction. Upon completion of the modifications, experiments will be undertaken to test the results of the present experiments.

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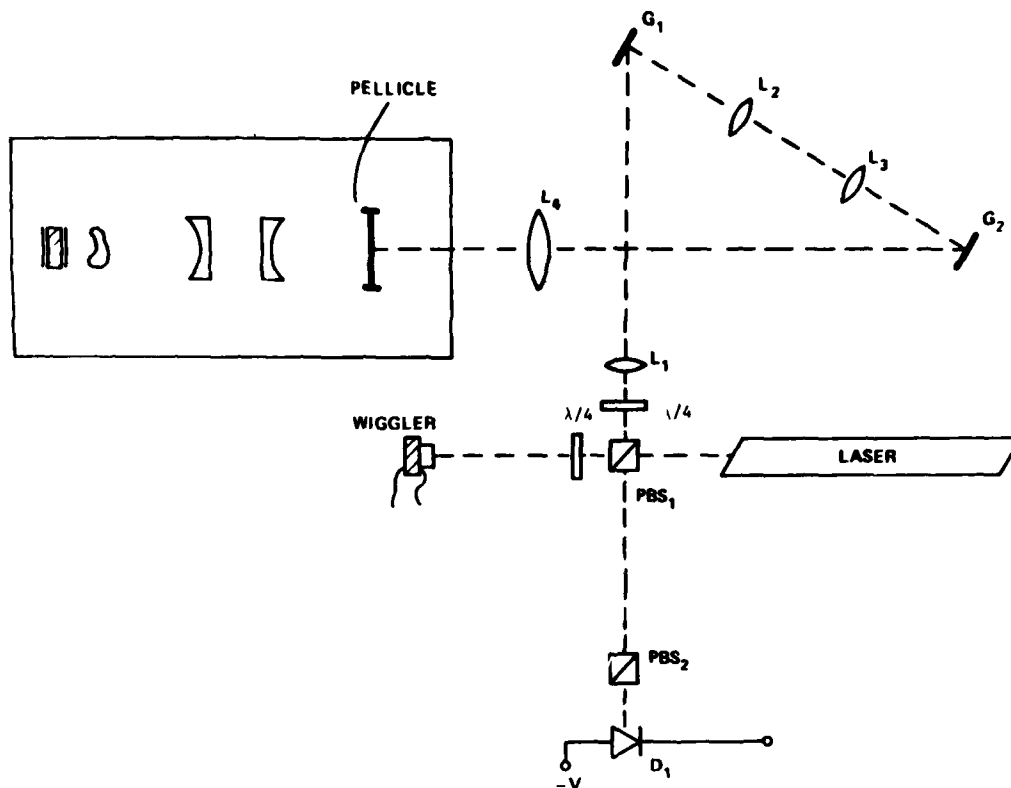


Figure 1. Basic schematic of Ultrasonovision. For complete description see Reference 1.

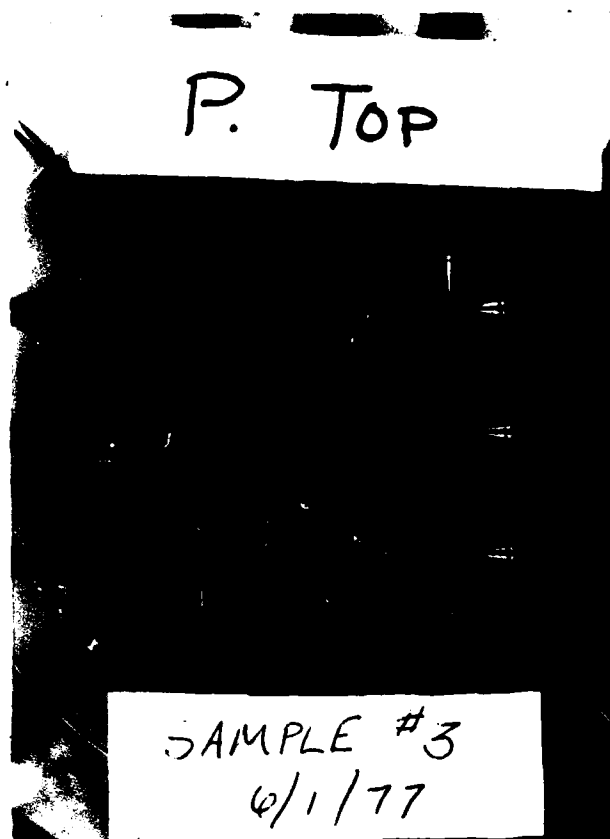


Figure 2. Sample of Bovine tissue mounted in special holder that provides acoustical as well as optical landmarks.

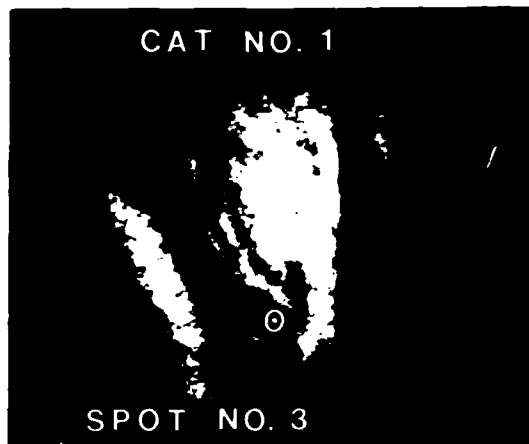
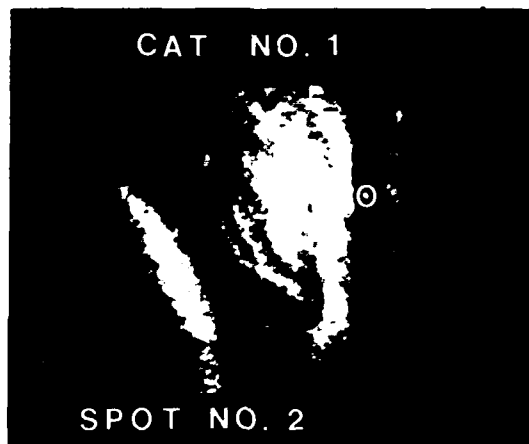


FIGURE 4 LIVING TISSUE

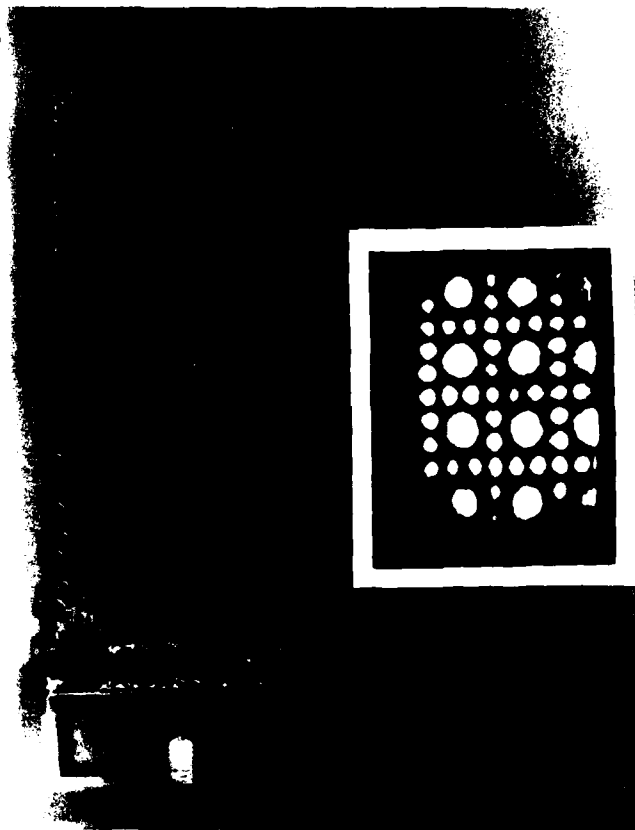


Figure 5. Left side of picture shows the focusing grid used for checking position of transducer lens system. The right side shows an acoustic image produced by sound transmitted through the grid.

CAT NO. 1

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-18-

FREQUENCY 1.75 MHz
TEMPERATURE 38° C

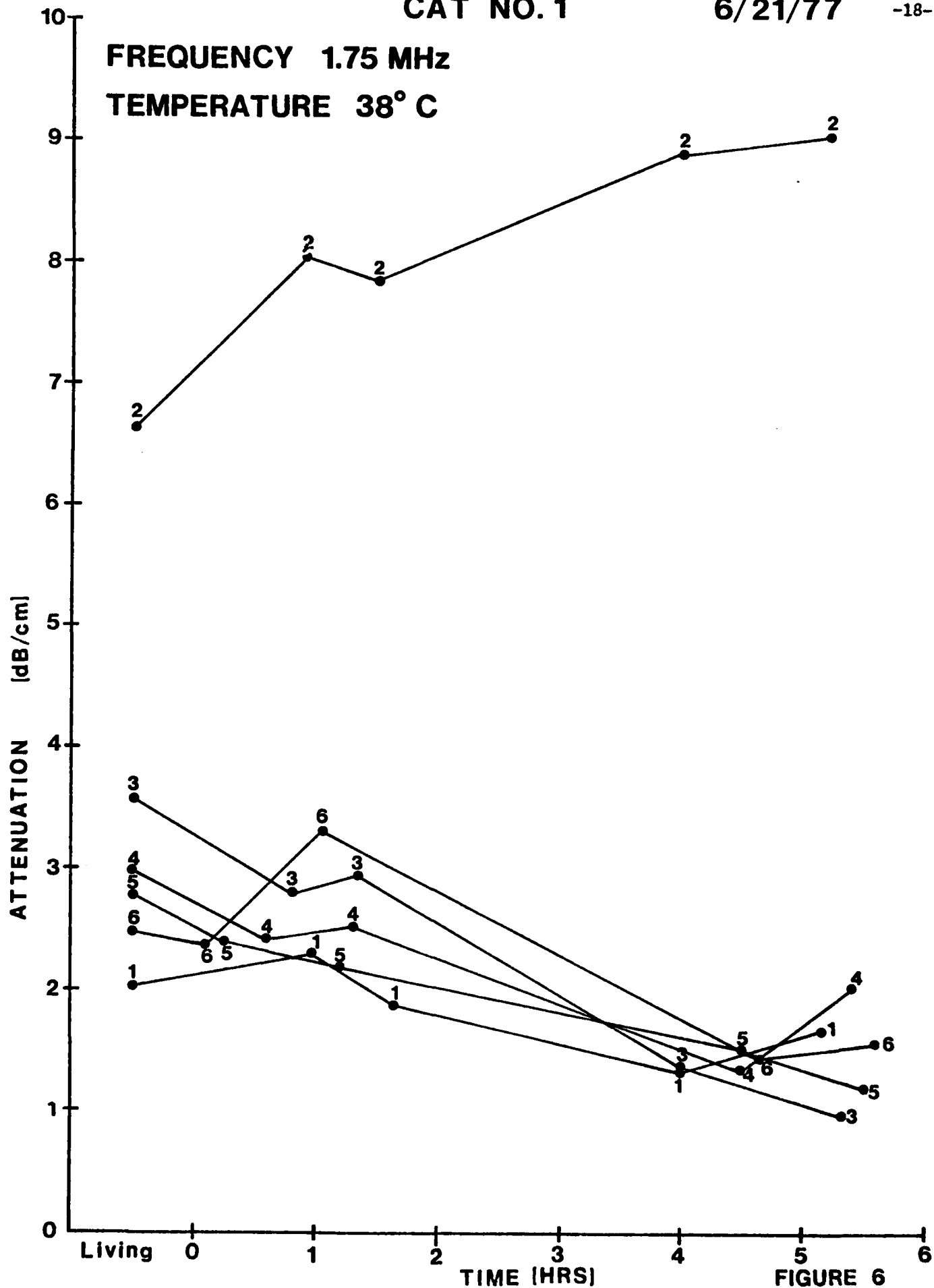
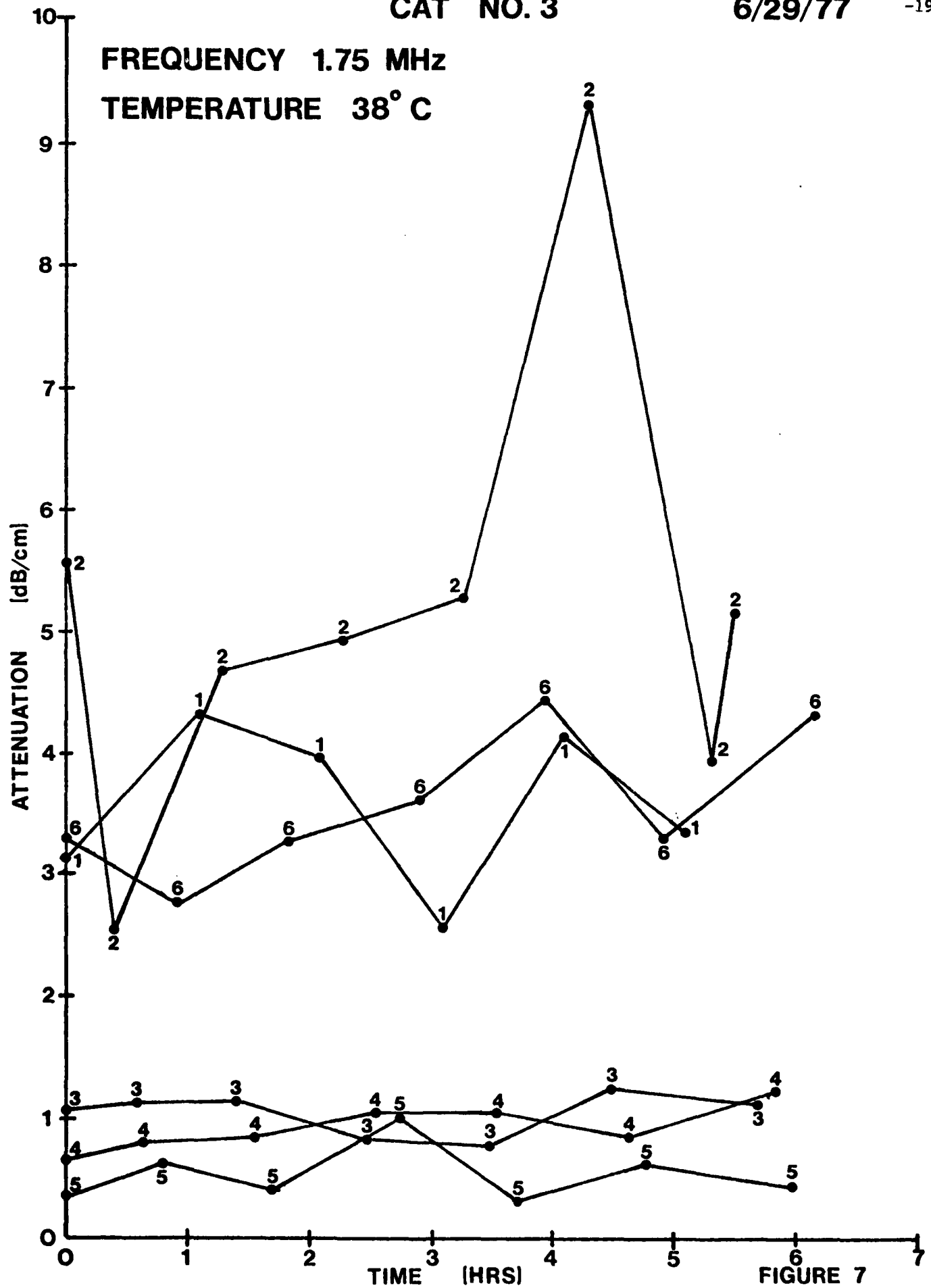
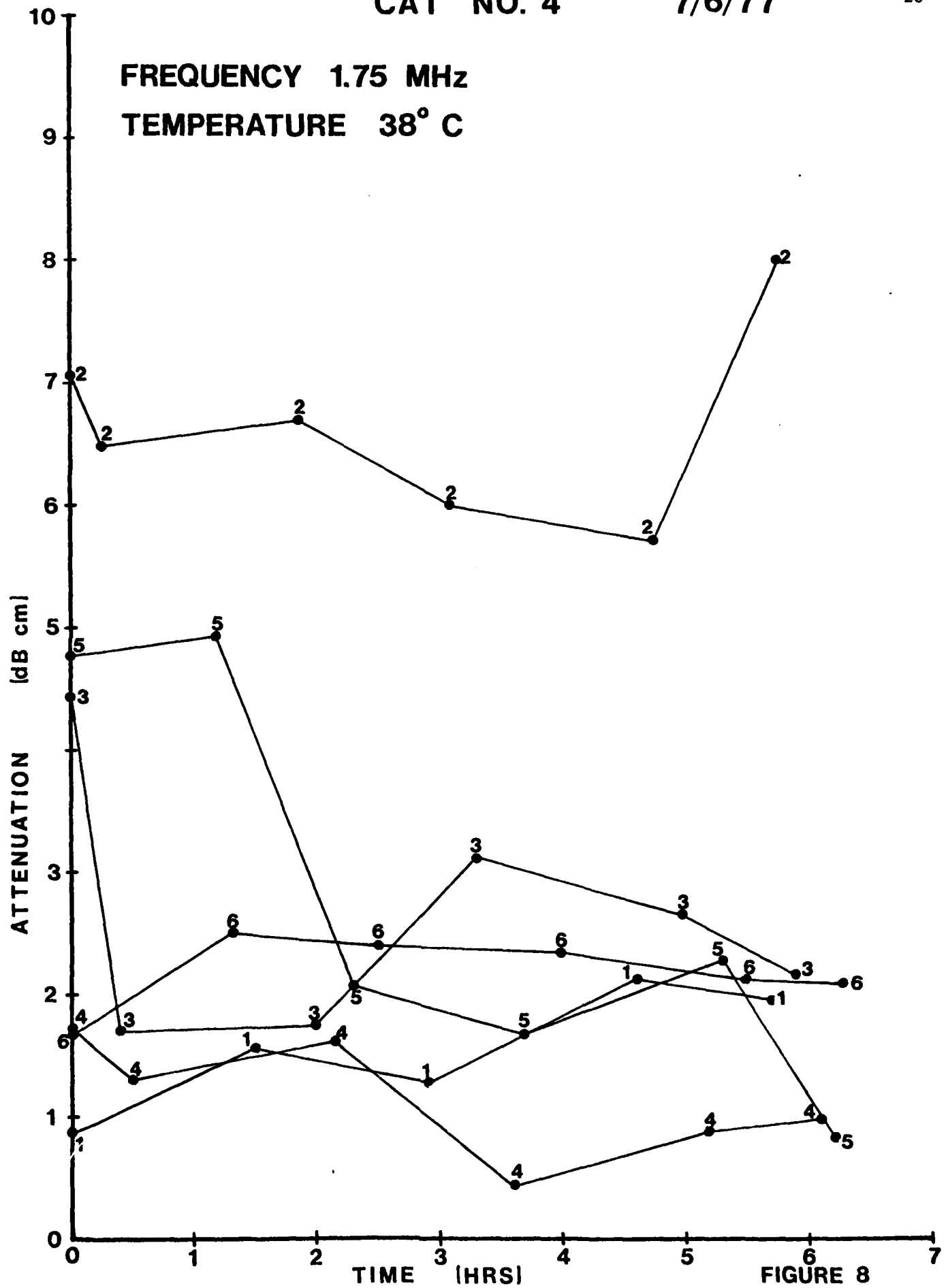


FIGURE 6



FREQUENCY 1.75 MHz
TEMPERATURE 38° C



FREQUENCY 1.75 MHz

TEMPERATURE 38°C

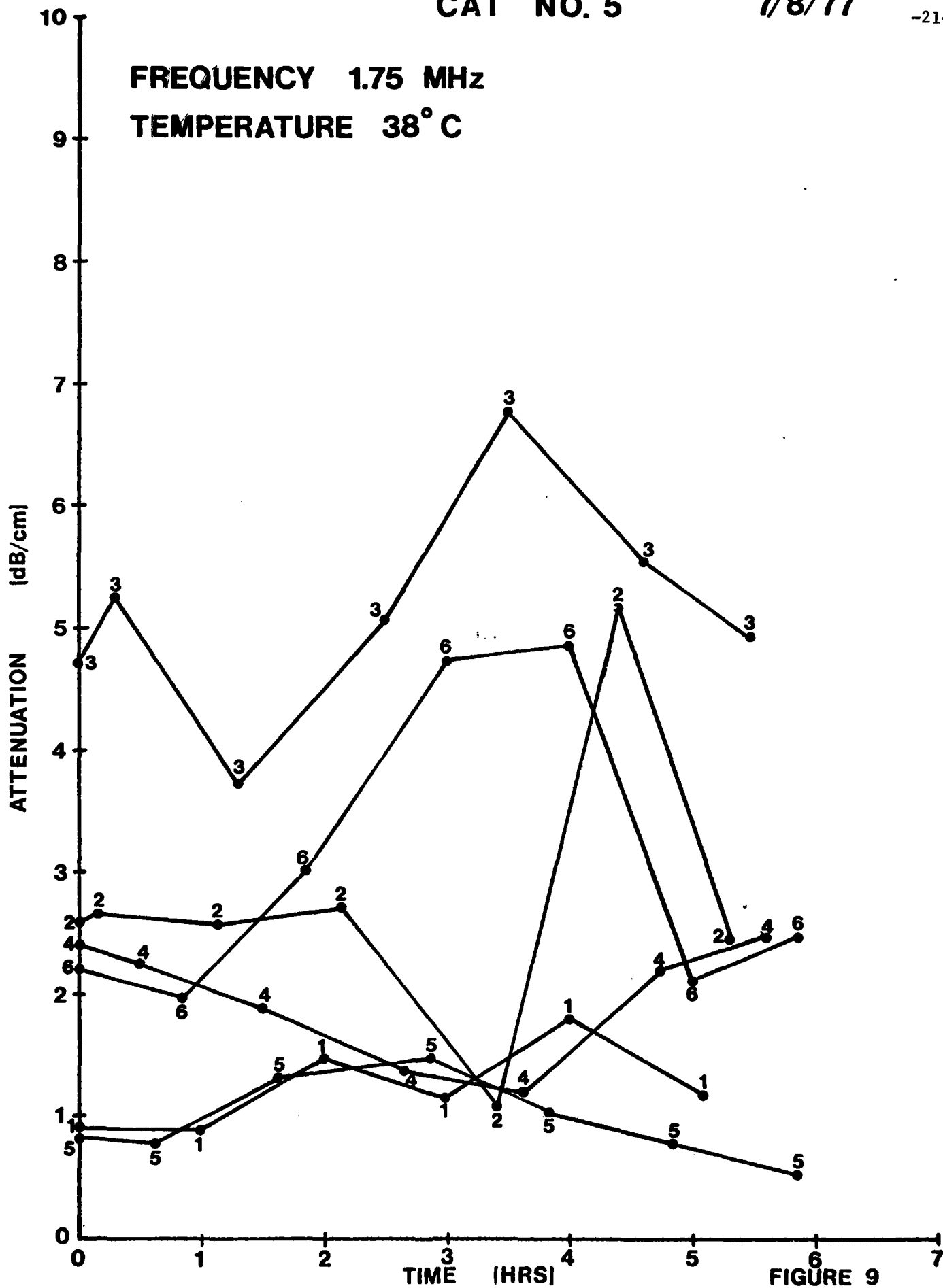


FIGURE 9

FREQUENCY 1.75 MHz
TEMPERATURE 38° C

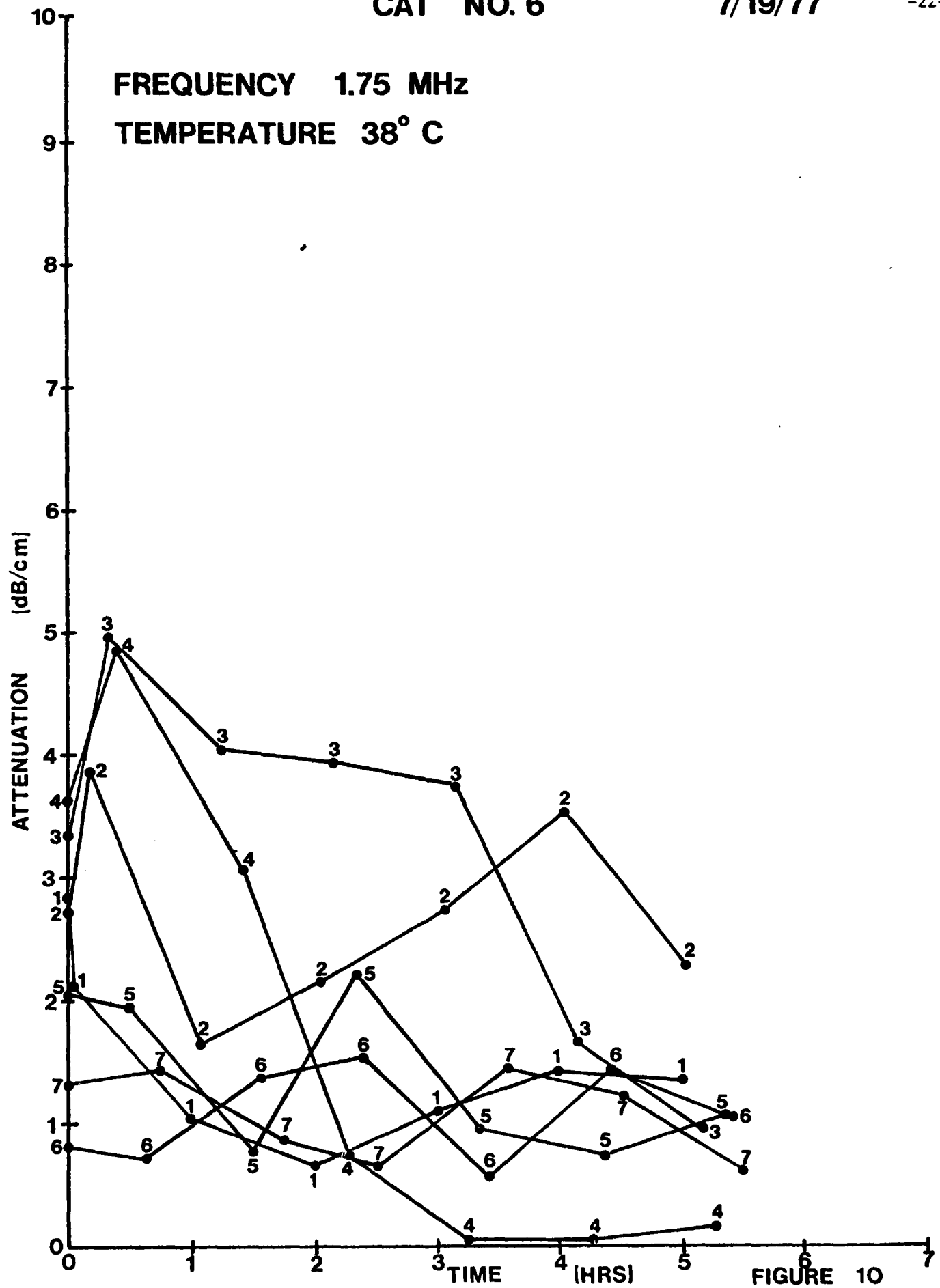


FIGURE 10

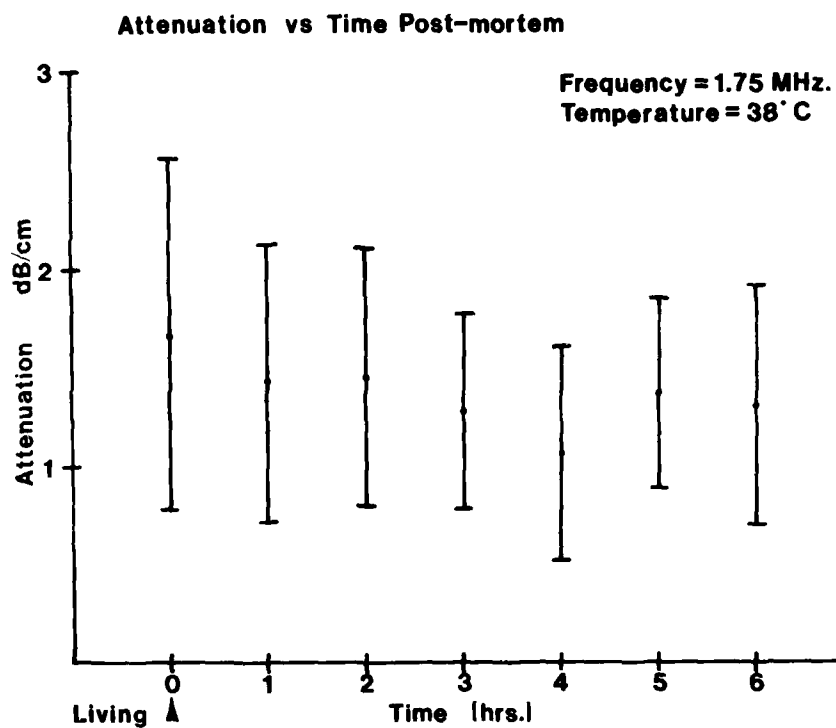


Figure 11. Plot of attenuation values versus time. The bars indicate standard deviation about the mean indicated by the small round dots on each line.

List of Publications
supported by this contract

- D. Feinstein, N.H. Farhat and A. Noordergraaf:
Acoustical Imaging Studies of Basic Tissue Models. San Diego
Biomedical Symp. 1975, pp 151 - 156.
- W.D. McNeely and A. Noordergraaf:
Differentiation between Normal and Necrotic Tissue Using Ultra-
sound. Abstracts First Mfg. World Federation for Ultrasound in
Med. and Biol., San Francisco, Cal., 1976.
- W.D. McNeely and A. Noordergraaf:
Differentiation Between Normal and Necrotic Tissue Using Ultra-
sound. In: Ultrasound in Medicine Vol. 3B. D. White and R.E.
Brown (eds.), pp. 1875 - 1881. Plenum Press, New York, 1977.
- W.B. McNeely and A. Noordergraaf:
In vivo Attenuation Measurement in Pre- and Postmortem Muscle
using Ultrasound. IEEE Trans. Sonics and Ultrasonics. In press.

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IN VIVO ATTENUATION MEASUREMENT IN PRE- AND POST-MORTEM MUSCLE
USING ULTRASOUND

W. D. McNeely

and

Abraham Noordergraaf, Ph.D.

Work supported by U.S. Army Surgeon General's Contract No. DAMD-17-74-C-4107.

Abstract

Changes in acoustic attenuation in cat muscle are measured in vivo for the transition period of living to six hours post-mortem. In the 0-4 hour period post-mortem the data indicated a decrease in attenuation, while in the 4-6 hour period the data suggested an increasing trend.

I. Introduction

The question of whether the acoustic properties of in vitro and in vivo tissue are equivalent is of major importance in the study of the acoustic properties of tissue. Such importance stems not only from the desire to correctly extrapolate results of measurements from the more easily determinable in vitro case to that element in vivo, but also from the desire to study those physiological characteristics of tissue which determine the acoustic properties of tissue. Previously, a number of groups have looked at acoustic propagation parameter changes in tissue with time post-mortem [1-7]. Frizzell et al. [1], using a radiation force detector, reported that in mouse and beef liver, the attenuation decreased about 10 percent over a period of 24 hours post-mortem. They concluded that the acoustic properties of freshly excised tissue were very close to those of in vivo tissue for a considerable length of time post-mortem. For a frequency of 1.8 MHz, they reported a range of attenuation values of 0.96 dB/cm for fresh tissue to 0.84 dB/cm for tissue 24 hours old. Hueter et al. [2], using a piezoelectric transmitter and receiver, reported a decrease of 0.36 dB/cm to 0.1 dB/cm in liver over a 48-hour period post-mortem. Bamber et al. [3] investigated attenuation with time post-mortem at 4, 5 and 6 MHz, and found little or no change (less than 10 percent) for up to 50 hours post-mortem. McNeely et al. [4], however, using a radiation force receiver (RCA Ultrasonovision), found that for bovine muscle starting at approximately 3 hours post-mortem to 48 hours post-mortem there was an overall decrease of about 3 to 5 dB/cm in attenuation.

The purpose of this study was to look at muscle before and after death to see if any measurable changes in the acoustic properties occur, as measured by

the RCA Ultrasonovision. Previous studies on various other tissues as described above indicated the attenuation change could be quite small. Muscle, however, differs from other body tissues in that 4-6 hours after death, rigor mortis occurs due to the lack of ATP in the muscle cells. ATP serves as a plasticizer in the actin-myosin bond, and as the ATP level falls the actin-myosin bond becomes quite rigid. The time required for onset of rigor mortis depends upon the supply of creatine phosphate and glycogen, both of which are part of the ATP chain, in the muscle at the time of death. Forster [8] reported that the average time for onset is 4-6 hours post-mortem and is dependent upon the ambient temperature, i.e., the higher the temperature, the faster the onset. In the present study, the attenuation coefficient in mammalian muscle is measured as a function of time post-mortem to investigate the effect of tissue preparation and condition on the acoustical properties.

Methods

Cats were chosen as the experimental animal for their size, availability, and ease of handling. The animals were first anesthetized using sodium pentobarbital. Next, the hair on the hind legs was removed by shaving. Then the animal was strapped to the restraint apparatus shown in Figure 1. A plastic bag of 1.5 mil polyethylene was placed around the lower assembly of the rack and the hind legs to prevent contamination of the tank. Such polyethylene was measured, using the Ultrasonovision, to have an attenuation of less than 0.1 dB at 1.75 MHz. Thus, its introduction into the measurement system will not skew the results. One leg was positioned normal to the axis of the ultrasound beam, with the acoustic beam focused on the area of muscle between the patella and the fibula (Figure 2). After insertion of the animal into the main tank

and filling the plastic bag with distilled water, the leg to be scanned was manually rubbed to remove air bubbles which would result in falsely high attenuation values. Water bath temperature was maintained at $38^{\circ} \pm 0.5^{\circ}\text{C}$.

A complete description of the RCA Ultrasonovision is given in [9], therefore, only a brief description will be given in this paper. The Ultrasonovision is an ultrasonic device working in the transmission mode. Acoustic energy from a transducer is focused onto a thin membrane receiver causing the face to ripple (Figure 3). The receiver, called the pellicle, is coated with gold making it a partial mirror to light waves. An interferometer scans the face of the pellicle and measures the slight deformation of the mirror face caused by the acoustic energy. The output of the interferometer is a phase shift whose amplitude is dependent upon the amount of rippling of the pellicle. This phase shift is converted into an electrical signal by a photodetector, and displayed on an oscilloscope as an intensity. The Ultrasonovision is capable of measuring sound intensities as small as 5 nWcm^{-2} ; thus, it is well suited for the experiments described in this paper.

A preliminary acoustic image was taken to provide a map for location of sites of interest (Figure 4). Usually, sites that contained both high and low attenuation extremes, i.e., 2.5 dB to 0.529 dB (Table 1), were chosen due to ease of identification. An initial attenuation reading was recorded at each of the pre-selected sites on the live animal; then an overdose of 5 ml sodium pentobarbital was administered to terminate the animal. Cessation of respiration usually occurred within 1 to 2 minutes. The time of death was arbitrarily made the same as the time of the overdose, and all subsequent readings were referred to as "post-mortem". The exact time of death is difficult to determine as different cells live varying lengths of time after loss of blood

flow. Each hour thereafter, the attenuation of each site was remeasured. Each reading consisted of recording the ultrasonic intensity with the animal in and out of the beam. For a single location, twelve readings were taken through the tissue and twelve of the reference path, i.e., no tissue. Twelve readings were necessary at each spot due to the inherent instability of the system, e.g., any vibration of the environment directly affected the intensity reading by randomly changing the amount of light that reached the detector. Small vibrations are normally overcome by the "wiggler", but the large vibrations in the laboratory tended to override the wiggler capability. Spatial noise from dirt on the lenses and pellicle also contributed to the instability of the system by causing varying degrees of reflection from the pellicle face and scattering light as it passed through each lens.

The attenuation coefficient is derived from experimental data by measuring the reference path, signal intensity and the tissue path intensity. The attenuation is given by the following equation:

$$\text{attenuation (dB)} = 20 \log \left(\frac{\text{tissue path intensity}}{\text{clear path intensity}} \right)$$

Measurements were performed using a focused 1.75 MHz transducer excited with an rf burst. For making individual attenuation measurements, the Ultrasonovision was equipped with a spot reading device that allowed the recording of attenuation on a single element of the image. The measurements were easily repeatable using a calibrated x-y grid system for exact pixel location. Accurate relocation of each spot of interest, and thus the same point within the tissue, was possible during the entire experiment, normally a total of 6 to 7 hours. Termination of the experiment was dependent upon the state of tissue decomposition.

Results

Upon completion of an experiment, the attenuation values were calculated for each spot and then plotted on a graph (Figure 5). Figure 5 shows the mean attenuation value for each preselected spot vs. the mean time it was recorded. Preliminary analysis indicated that the data followed the pattern predicted by in vitro experiments using bovine muscle (McNeely et al., [4]); that is, attenuation decreased with time post-mortem. Data were then collected on four additional animals. Figure 6 shows a composite plot of mean attenuation vs. mean time. The bars indicate standard deviation limits, while the small dots indicate mean value.

If the data (Table 1, Figure 6) are divided into two stages, i.e., living to 4 hours post-mortem, and 4 hours post-mortem to 6-7 hours post-mortem, then separate analysis of each part may be performed. By applying a linear regression "best fit" to the 0-4 hours data and the 4-6 hours data, reasonably good fit values of r^2 result. For the 0-4 hours period, an r^2 of 0.93, (r of 0.96) is calculated, and for the 4-6 hours period, an r^2 of 0.98, (r of 0.99) results. Application of a critical value test of the r values will determine if any correlation exists between time and the data points in the form of a significance level, since for five points or more, a critical r value of 0.878 indicates a statistically significant correlation ($p \leq 0.05$). Therefore, for the 0-4 hours interval, there is a significant correlation. For the interval of 4-6 hours post-mortem, there are not enough points to apply the critical value test. These results would seem to indicate that there are two different mechanisms involved in affecting the acoustic properties of tissue immediately following death.

Discussion

One mechanism in the 0-4 hour period post-mortem causes a reduction in the attenuation. This reduction could be explained by pooling of blood at death in the lowest body parts, away from the scanned region. The upper portion of the leg was examined, and it is possible that due to the animal's position (Figure 1), blood moved into the lower portion of the leg. Dussik and Fritch [7] found a similar occurrence when they restricted the circulation in a limb. As in the present experiments, a slight decrease in attenuation was measured.

In the 4-6 hour period post-mortem an increase in attenuation, possibly due to the onset of rigor mortis, occurs. Dussik and Fritch [7] reported similar results when the muscle tonus was changed; however, they were not able to measure both the decrease and subsequent increase in the same subject as the two effects tended to cancel. In our experiments, the method of measurement was more sensitive and the experimental design different, allowing differentiation between the two effects.

Within the measurement accuracy of the Ultrasonovision, there is a decrease in attenuation in the 0-4 hours post-mortem range, while in the 4-6 hour interval, the attenuation appears to be increasing.

Conclusion

Ultrasonic attenuation has been measured in the leg muscle of the cat using the RCA Ultrasonovision. Due to its sensitivity, the Ultrasonovision has been able to measure significant changes in attenuation that occur in the first six hours following death. These results indicate that there are at

least two mechanisms affecting acoustic attenuation during the time immediately following death. Therefore, in future tissue characterization extrapolation from the in vitro case to the in vivo case, some modification must be made to take these effects into account.

Acknowledgment

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-All-

TABLE A

Cat No.	Measurement point	<u>Attenuation (dB/cm)</u> (minutes post-mortem)					
1.	1	<u>2.025</u>	<u>2.299</u>	<u>1.8813</u>	<u>1.352</u>	<u>1.683</u>	
		living	(55)	(95)	(180)	(310)	
	2	<u>2.995</u>	<u>2.424</u>	<u>2.5264</u>	<u>1.354</u>	<u>2.0354</u>	
		living	(38)	(75)	(270)	(325)	
	3	<u>2.775</u>	<u>2.40</u>	<u>2.14</u>	<u>1.529</u>	<u>1.226</u>	
		living	(10)	(70)	(275)	(330)	
	1	<u>1.0827</u>	<u>1.12</u>	<u>1.125</u>	<u>0.805</u>	<u>0.795</u>	<u>1.233</u>
		living	(30)	(82)	(143)	(203)	(265)
	2	<u>0.66</u>	<u>0.805</u>	<u>0.8425</u>	<u>1.07</u>	<u>1.075</u>	<u>0.8435</u>
		living	(40)	(93)	(154)	(213)	(278)
2.	3	<u>0.324</u>	<u>0.62</u>	<u>0.4211</u>	<u>1.00</u>	<u>0.305</u>	<u>0.6145</u>
		living	(48)	(102)	(165)	(223)	(286)
	1	<u>2.65</u>	<u>0.8815</u>	<u>1.5663</u>	<u>1.2907</u>	<u>2.12145</u>	<u>1.938</u>
		living	(5)	(90)	(175)	(275)	(340)
	2	<u>1.725</u>	<u>1.305</u>	<u>1.625</u>	<u>0.4365</u>	<u>0.89</u>	<u>0.955</u>
		living	(40)	(130)	(215)	(310)	(365)
	3	<u>0.324</u>	<u>0.62</u>	<u>0.4211</u>	<u>1.00</u>	<u>0.305</u>	<u>0.6145</u>
		living	(48)	(102)	(165)	(223)	(286)
	1	<u>2.65</u>	<u>0.8815</u>	<u>1.5663</u>	<u>1.2907</u>	<u>2.12145</u>	<u>1.938</u>
		living	(5)	(90)	(175)	(275)	(340)
3.	2	<u>1.725</u>	<u>1.305</u>	<u>1.625</u>	<u>0.4365</u>	<u>0.89</u>	<u>0.955</u>
		living	(40)	(130)	(215)	(310)	(365)
	3	<u>0.324</u>	<u>0.62</u>	<u>0.4211</u>	<u>1.00</u>	<u>0.305</u>	<u>0.6145</u>
		living	(48)	(102)	(165)	(223)	(286)
	1	<u>2.65</u>	<u>0.8815</u>	<u>1.5663</u>	<u>1.2907</u>	<u>2.12145</u>	<u>1.938</u>
		living	(5)	(90)	(175)	(275)	(340)
	2	<u>1.725</u>	<u>1.305</u>	<u>1.625</u>	<u>0.4365</u>	<u>0.89</u>	<u>0.955</u>
		living	(40)	(130)	(215)	(310)	(365)
	3	<u>0.324</u>	<u>0.62</u>	<u>0.4211</u>	<u>1.00</u>	<u>0.305</u>	<u>0.6145</u>
		living	(48)	(102)	(165)	(223)	(286)

	3	<u>1.6935</u> living	<u>2.503</u> (80)	<u>2.39</u> (150)	<u>2.345</u> (240)	<u>2.125</u> (330)	<u>2.06</u> (380)	
4.	1	<u>0.988</u> living	<u>0.90</u> (3)	<u>0.898</u> (65)	<u>1.475</u> (125)	<u>1.14</u> (190)	<u>1.82</u> (250)	<u>1.095</u> (305)
	2	<u>2.41</u> living	<u>2.25</u> (30)	<u>1.875</u> (90)	<u>1.254</u> (160)	<u>1.1978</u> (220)	<u>1.095</u> (285)	<u>2.455</u> (335)
	3	<u>0.8375</u> living	<u>0.782</u> (40)	<u>1.31</u> (100)	<u>1.495</u> (170)	<u>1.0335</u> (230)	<u>0.778</u> (290)	<u>0.505</u> (345)
5.	1	<u>2.886</u> living	<u>2.1199</u> (3)	<u>1.05</u> (65)	<u>0.6808</u> (125)	<u>1.115</u> (185)	<u>1.408</u> (245)	<u>1.36</u> (305)
	2	<u>0.8195</u> living	<u>0.72965</u> (40)	<u>1.38</u> (75)	<u>1.564</u> (145)	<u>0.579</u> (205)	<u>1.467</u> (265)	<u>1.052</u> (325)
	3	<u>1.3245</u> living	<u>1.45</u> (45)	<u>0.883</u> (105)	<u>0.65</u> (150)	<u>1.448</u> (215)	<u>1.225</u> (270)	<u>0.603</u> (330)

Figure Legends

- Figure A1 Cat mounted in restraint apparatus inserted into Ultrasonovision tank.
- Figure A2 Close-up of cat's lower leg. Screws serve as both acoustical and optical markers.
- Figure A3 Line drawing showing relationship of components of Ultrasonovision.
- Figure A4 Series of acoustic images - living tissue to 6 hours post-mortem.
- Figure A5 Plot of attenuation values for cat No. 1, Point No. 2 taken through bone.
- Figure A6 Composite plot of attenuation values for five cats. Points used were through muscle.



Figure A1



Figure A2

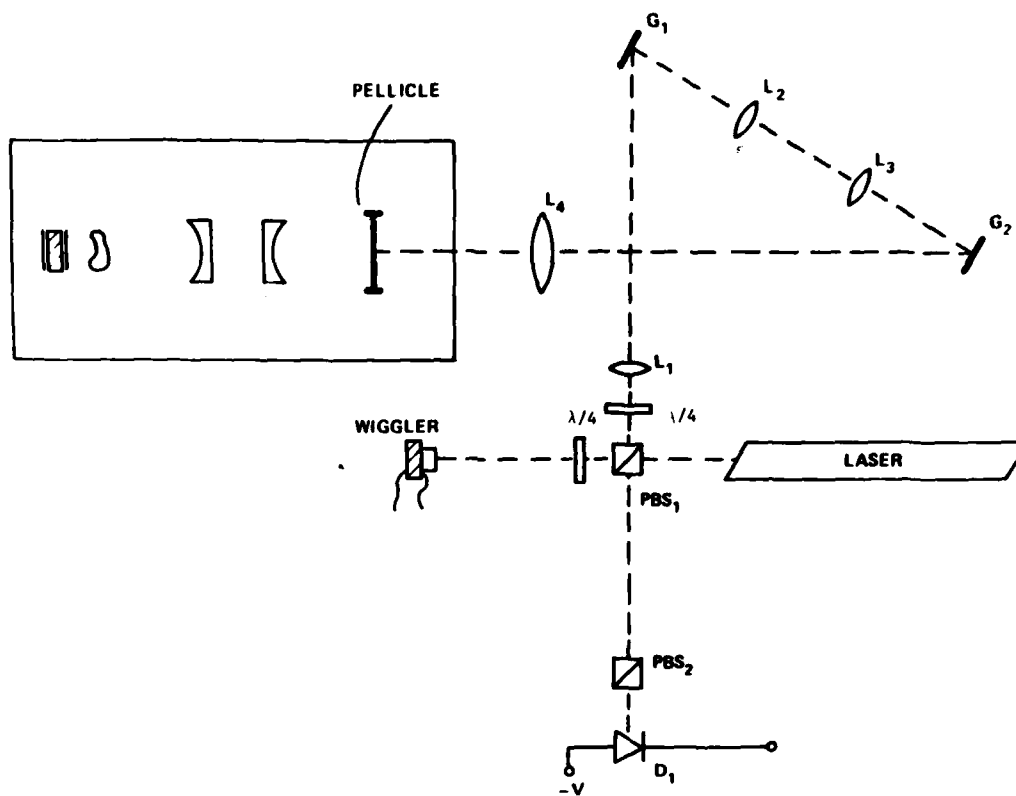


Figure A3

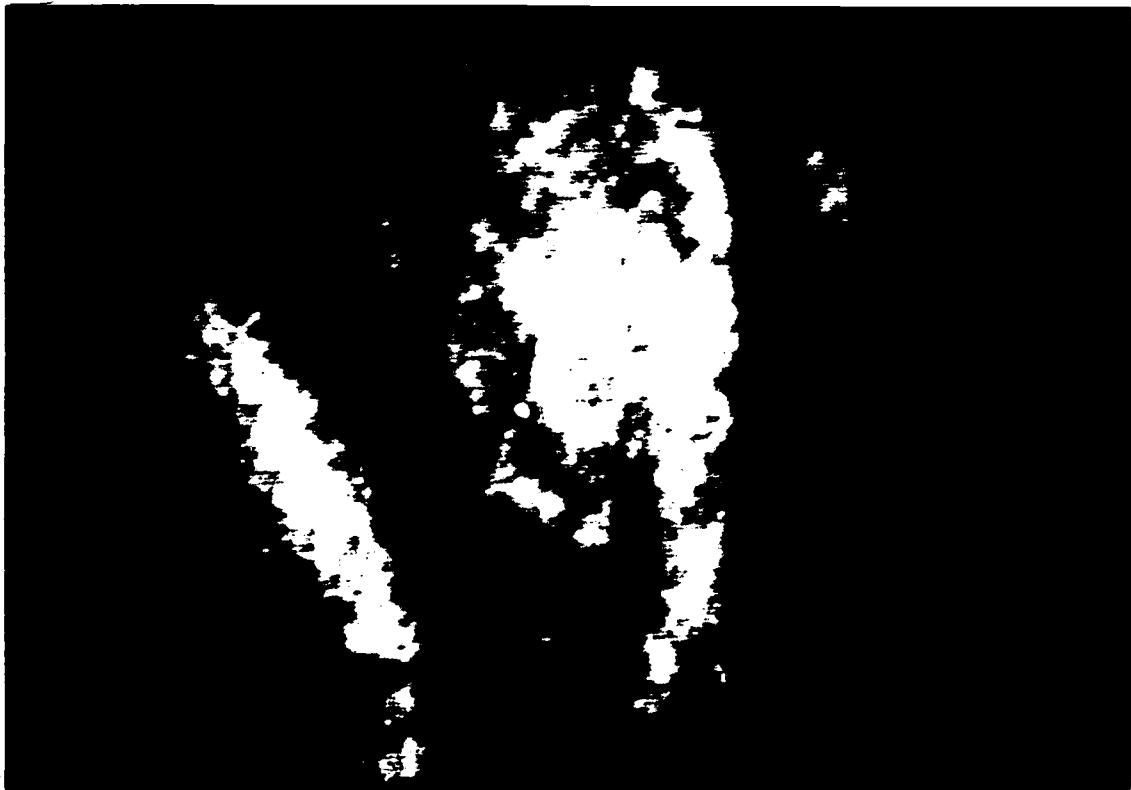


Figure A4

Living



Figure A4
6 Hours Post-mortem

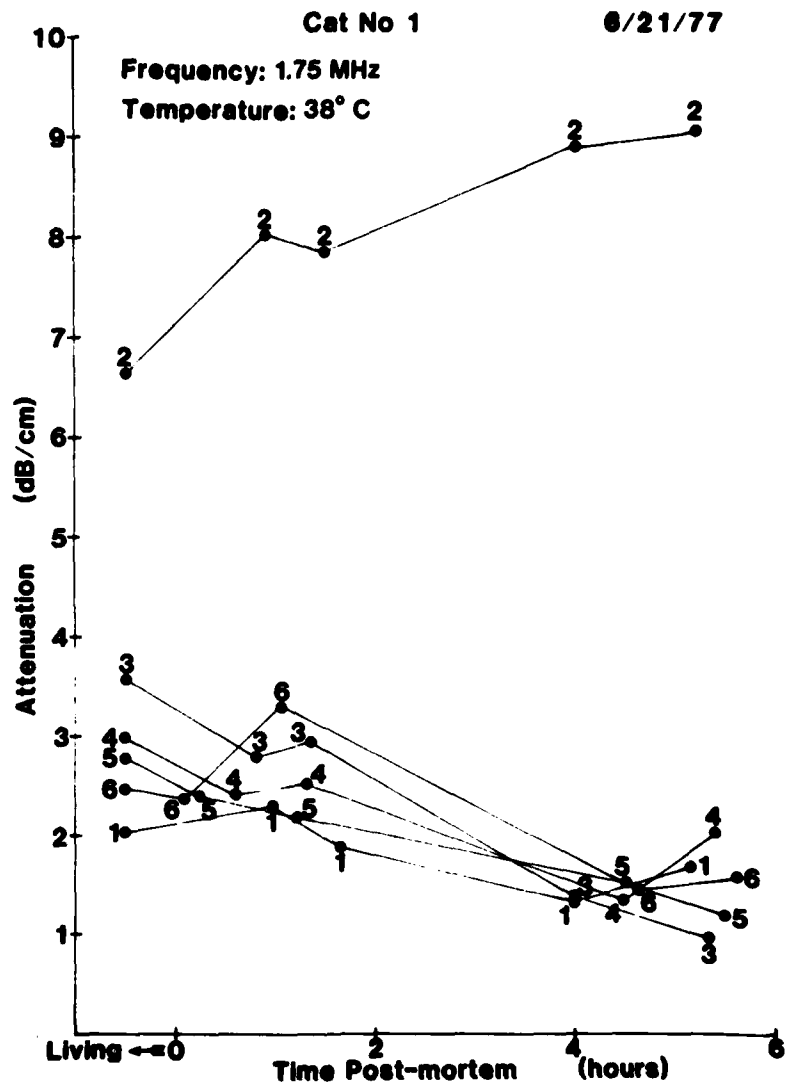


Figure A5

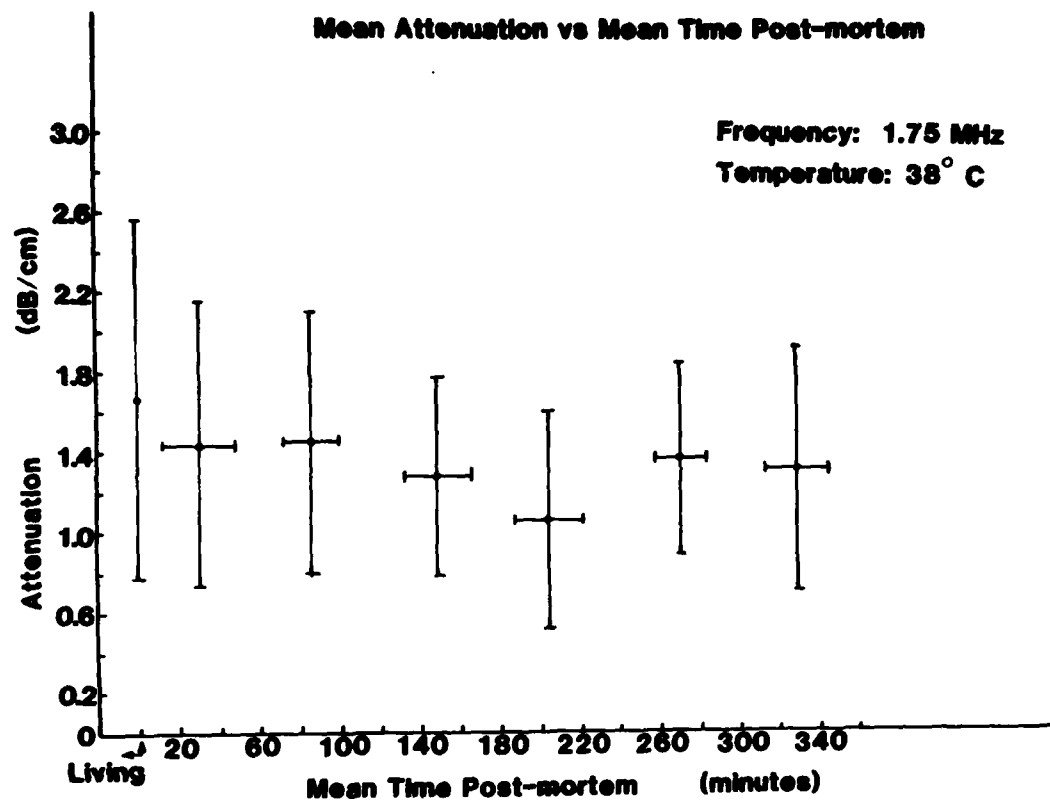


Figure A6

